		simulations", but it is only used for a 'vbal' equation (see below). While it does not matter for the model, there is no constraint preventing this from being a negative number (if sum of perfused tissue fractions is near greater than 1 during random draws). Constraint may be in MC scripts.
VMAX, VMAXLU, VMAXKD	Metabolic rates of liver, lung, kidney	Equation correct (BW^3/4 scale)
KFLU	Pseudo first-order rate	Equation correct (scaled by BW)
CIX	Ideal gas constant and molecular weight conversion	Equation correct (multiplied by MW/24450). Note: No comment is associated with this line. Should state units conversion (ppm to mg/L)
CI	Inhalation exposure control	Correct (multiplied by binary EXPPULSE switch). Verified by running model to debug prior mistake with forcing function/exposure scripts.
Tissue venous concentrations	Mass of chemical in tissue to venous concentration	Correct (amount/(volume x partition)) for each tissue (tissue/amount/partition indices match).
CPU	Pulmonary mass balance (QP*CI+(QF*CVF + QL*CVL + QS*CVS + QR*CVR + QK*CVK))/(QP/PB+QC)	Equation correct. Note: An optional addition could be a dead-space fraction, if needed to account for model/data discrepancies for the given QC and QP numbers.
CX	Exhaled concentration = CPU/PB	Equation correct.
CV	CV = (QF*CVF + QL*CVL + QS*CVS + QR*CVR + QK*CVK)/QC	Correct. Note: this parameter not used in code, and is not an output. Typically, it is used to condense the pulmonary mass balance equation, or as a biomarker.
CPUM	Units conversion for CPU.	Parameter not used in code, and not an output. Unclear of the purpose.
RAI, RAX	Inhalation/exhalation rate equations and mass balance	Equations correct
RAM, RAMLU, RAMK	Rate equations for metabolism in liver, lung, kidney	Correct (reaction concentration is correctly-indexed venous blood

		concentration for all, using post-BW scaled parameters).
RALU, RAL, RAK, RAS, RAR, RAF	Rate equations for mass balance lung, liver, kidney, slowly perfused, rapidly perfused, fat	Correct. Lung applies CPU (blood concentration at air/blood exchange) input and QC flow. Other systemic organs apply lung venous flow as input, and correctly-indexed venous streams and metabolism as outputs.
Outputs bloc		
MASBAL	MASBAL = AI - AX - (AL+AM+AMLU+ALU+AK+AMK+AS+AR+AF)	Correct (overall mass balance not missing any tissues/sources/sinks)
Concentrations calculations	Concentrations in tissues and for plots	Correct
Dose metrics	Definitions of AMP, AMPLU, AMPK (units conversions, and cumulative time averaging)	Correct, however, final units should be stated as comments.
Blood/tissue balances	Error checks on total blood and volume fractions	Correct

Table 2. Quality review of invitro.csl

File or variable	Definition	Notes and determination
	INITIAL bloc	
Model parameters	VMAX1, KM1, RLOSS, VK, P1, A10, VVIAL, VMED, VAIR=VVIAL-VMED, PROT, VINJ	Comments and definitions are poorly documented. **1)** VINJ says "based on Matt email" but the last paragraph of Himmelstein et al. (2004) p. 19 gives 400 uL as sample volume for CP oxidation experiments, which differs from 200 uL used for CEO experiments. In V_human.m VINJ is set to 0.0003858 L. An explanation is needed for how VINJ was measured so precisely for humans, and confirmation that it differs from other experiments described in the same paper. Otherwise it should be 0.004 uL for all Himmelstein et al. (2004) data. **2)** Yang et al. (2012), section 2.1.3, states that 200 uL samples were used for those experiments. **3)** VVIAL differs from default (0.01165 L) in the following files: V_kidney.m (0.01163); V_human.m (0.0119573). While the variation likely has minimal impact, a single value should be used in the absence of specific data.
Time variables and timing commands	TF, TI, VINJ, TSTOP, POINTS, CINT, TS=TF	Sampling is "disruptive" (in the experiment, sampling the headspace affects the mass balance). The simulated timing should match the experimental condition, but where different replicates used different sample times, a representative average would be sufficient (i.e., time of first sample should be average of initial times from replicates). The total number of samples should accurately reflect those taken from each incubation vial.

Initial conditions	CA10=A10/(VAIR+P1*VMED), CM10=CA10*P1, CA1=CA10, CM1=CM10, A1I=0.	Initial conditions would need re-structuring if an alternative 2-compartment model is applied (see below)
DYNAMIC/DERIVATIVE bloc		
Integration and models	Three differential rates (although only a 1-compartment mass balance is performed, which includes a differential loss term) !CD KINETICS (umoles/hr) R1M=(VMAX1*CM1)/(KM1+CM1)*PROT RRLUNGVK=VK*CM1 RRLoss = RLOSS*CM1 A1M=INTEG(R1M,0.) ARLUNGVK=INTEG(RRLUNGVK,0.) ARLOSS = INTEG(RRLoss,0.) CA1=(A10-A1M-ARLUNGVK-A1I-ARLOSS)/(VAIR+VMED*P1) CM1=CA1*P1 A1=CA1*VAIR+CM1*VMED	The model assumes instantaneous steady-state in the liquid phase (applying only the media/air partition coefficient for the chemical). Model-predicted headspace concentrations were found to be significantly different if instead applying a more realistic 2-compartment system (assuming concentration-driven mass transport). Estimation of Km would likely be different if model was optimized assuming 2 compartments. Hence, a reasonable estimate for a mass transfer term between liquid gas phase is needed to develop a model that accurately reflects the physical system. Based on example simulations, equilibration must occur in much less than 1 min in order for the assumption to be valid.
DISCRETE bloc		
Discrete events affecting mass balance (doses, sampling, etc).	Contains the routine for mass loss due to sampling A1I=A1I+CA1*VINJ SCHEDULE step .AT. TS+TI TS=TS+TI	See comments under “time variables and timing commands”.

Other notes:

VAIR is calculated in the .csl code (VAIR=VVIAL-VMED) based on the CONSTANT values VVIAL and VMED (even if they are not set to defaults). However, this calculation also appears in most of the script (*.m) files. To avoid confusion/redundancy, the line VAIR=VVIAL-VMED should be removed from script files.

Table 3. Check of metabolic parameters (in-vitro) against Yang et al. (2012) and Himmelstein et al. (2004). *[Currently awaiting decisions regarding 2-compartment model]*

File name	Metabolc parameters set	Disp.
V_human.m (PROT=1.0)	VMAX1=0.054; KM1=0.45; VK = 0.0;	
	VMAX1=0.0; KM1=0.0; VK = 0.9/1000;	
	VMAX1=0.405/1000; KM1=0.45; VK = 0;	
V_kidney.m (PROT varies between 2.0 and 3.0 between runs)	VMAX1=0.0027; KM1=0.92; VK = 0.0;	
	VMAX1=0.00226; KM1=0.69; VK = 0;	
	VMAX1=0.00177; KM1=0.37; VK = 0.0;	
	VMAX1=0.0027; KM1=0.69; VK = 0;	
	VMAX1=0.01; KM1=0.5; VK = 0.0;	
	VMAX1=0.01; KM1=0.95; VK = 0;	
	VMAX1=0.00004; KM1=1.7; VK = 0.0;	
	VMAX1=0.0001; KM1=0.95; VK = 0;	
VFM_liver.m (PROT=1)	VMAX1=0.09; KM1=0.53; VK = 0;	
	VMAX1=0.12; KM1=0.95; VK = 0;	
VFM_lung.m (PROT=1)	VMAX1=0.025; KM1=2.78; VK = 0;	
	VMAX1=0.01; KM1=0.95; VK = 0;	
VFR_liver.m (PROT=1)	VMAX1=0.068; KM1=0.82; VK = 0.0;	
	VMAX1=0.055; KM1=0.69; VK = 0;	
VFR_lung.m (PROT=1)	VMAX1=0.0; KM1=0.0; VK = 1.2/1000;	
	VMAX1=1.02/1000; KM1=0.69; VK = 0;	
VMM_liver.m (PROT=1)	VMAX1=0.26; KM1=1.36; VK = 0.0;	
	VMAX1=0.21; KM1=0.95; VK = 0;	
VMM_lung.m (PROT=1)	VMAX1=0.13; KM1=2.0; VK = 0.0;	
	VMAX1=0.05; KM1=0.95; VK = 0;	
VMR_liver.m (PROT=1)	VMAX1=0.077; KM1=0.56; VK = 0.0;	
	VMAX1=0.086; KM1=0.69; VK = 0;	
VMR_lung.m (PROT=1)	VMAX1=0.0; KM1=0.0; VK = 0.9/1000;	
	VMAX1=1.86/1000; KM1=0.69; VK = 0;	

Message

From: Harvey Clewell [HClewell@ramboll.com]
Sent: 8/31/2018 3:40:52 PM
To: Schlosser, Paul [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=121cf759d94e4f08afde0ceb646e711b-Schlosser, Paul]; Jerry Campbell [JCampbell@ramboll.com]
CC: Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]
Subject: RE: Chloroprene In Vitro model

In case you don't have it handy, here is the paper Matt referred to.

Harvey Clewell
Principal Consultant

D +1 (919) 765-8025
M +1 (919) 4524279
hclewell@ramboll.com

From: HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Sent: Friday, August 31, 2018 7:37 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Jerry Campbell <jcampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Paul,

Early in vitro work used a Buker shaker the kind of which we also had at CIIT, and was used for 1,3-butadiene in vitro metabolism as well as for all in vitro blood-to-air gas partitioning work pioneered by Gargas at the WPAFB.

We subsequently switched to a Gerstel head space/incubator/mixer auto sampler attached to the HP GC/MSD (see attached photo) http://www.gerstel.com/en/MPS-Agitator-Incubator-Stirrer.htm. All incubations were conducted with a ~1:10 liquid to air ratio (1 mL in 10 mL vial). My understanding is these facilitates rapid equilibration. Any preincubation time was conducted absent metabolizing protein or NADP. A lot of the initial methods were worked out and published in 2001. Sampling at 12 minute intervals was conducted but as I recall, the start times were staggered to fill in for a more continuous curve using multiple incubation vials.

Hope this helps.

I am out of the office today. Back Tuesday.

Matthew Himmelstein
DuPont Haskell Global Centers
Phone 302 451 4537

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Thursday, August 30, 2018 9:54 AM
To: Jerry Campbell <JCampbell@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: [EXTERNAL] RE: Chloroprene In Vitro model

The other possible check is if experiments were run to check linearity of the initial slope with microsome concentration. I'm pretty sure that if mass transfer resistance is at play, you would see a less-than a doubling of the elimination rate when microsome concentration was doubled.

-Paul

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Thursday, August 30, 2018 9:41 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Paul,

There were control experiment data in the 2004 in vitro paper – Figure 3A.

Jerry Campbell
Managing Consultant

D 919-765-8022
jcampbell@ramboll.com

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Thursday, August 30, 2018 9:39 AM
To: HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: FW: Chloroprene In Vitro model

Matt,

As a follow-up, see the email from Jerry below… You can follow the thread below that if you wish!

While the question Jerry asks is if you had gentle mixing going on, the information we really need is on the mass-transfer rate under those conditions. Did you ever run control experiments like the plot below, for another chemical if not CP?

Jerry: note that my incubations were also in a shaker, but I think the amount of surface motion would be dampened considerably in a smaller vial.

-Paul

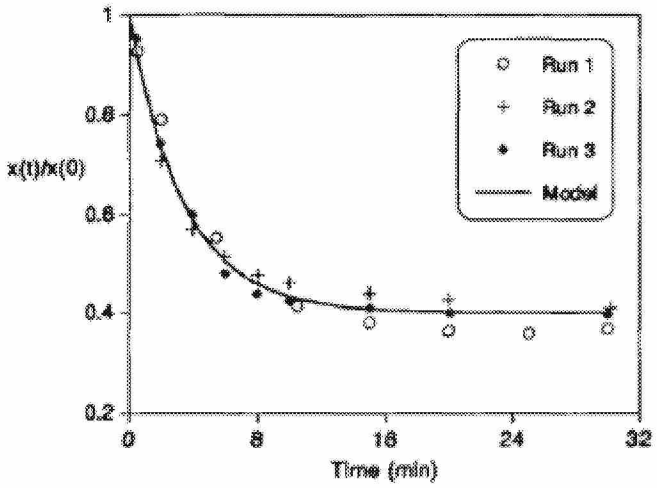


Fig. 3. Partitioning of benzene from liquid phase, into gas phase, in the absence of microsomes under incubation conditions (37°C shaker); $x(t)$ = concentration of benzene in the liquid phase at time = t; $x(0)$ = concentration of benzene in the liquid phase at time = 0. Different initial values, $x(0)$, were used for each run. The model is as depicted in Figure 1 with the rates of biotransformation ($r_1 - r_3$) set to zero.

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Thursday, August 30, 2018 9:10 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Harvey Clewell <HClewell@ramboll.com>
Subject: RE: Chloroprene In Vitro model

Paul,

Equilibration time question you might want to ask Matt is did they have a shaking sample heater for the headspace vials on their robot? I'm pretty sure they did. The version I had at UGA (was same system sold under another name) had controlled orbital shaking heater that could be set to very slow rotations per min (less than 10/min if I remember correctly) to provide gentle motion. It doesn't say explicitly in the method but it is possible that they used slow rotation to increase surface turnover and decrease liquid equilibration time. We generally used an orbital shaking water bath for non-volatile microsomal metabolism so I wouldn't be surprised if they did include some motion with their analysis too. .

Jerry Campbell
Managing Consultant

D 919-765-8022
jcampbell@ramboll.com

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Wednesday, August 29, 2018 4:08 PM
To: Jerry Campbell <JCampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Sasso, Alan <Sasso.Alan@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>
Subject: RE: Chloroprene In Vitro model

P.S. If there are data for another chemical where the equilibration rate was measured, those could be used with an adjustment of the PC. But it has to be 100x or more faster than what I measured to give results that are indistinguishable from the model where it's assumed to be instantaneous, and that seems unlikely to me.

Or if not, but someone has the system running for other chemicals, it's only a handful of experiments, no tissues.

-Paul

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Wednesday, August 29, 2018 3:17 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Harvey Clewell <HClewell@ramboll.com>; Sasso, Alan <Sasso.Alan@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>
Subject: RE: Chloroprene In Vitro model

In essence, there was only one sample scheme (every 0.2 hr or 12 min) but I think it may be more complicated than you have coded. It was an automated system – older version of the combi-pal autosampler. In the more highly sampled incubations (2004 paper in vitro paper), Matt reports that up to 5 vials were used to complete a time-course. So, while there was a mass of sample removed at each time, it wasn't linear throughout the whole run. He does state that samples were taken at 12 min intervals which coincides with the 1 vial system data in the female mouse and rat studies. The question is, can we assume that the 0.2 interval samples in the more highly sampled time-course is from a standardized staggered vial system:

Vial 1: 0, 0.2, 0.4, etc…
Vial 2: 0.05, 0.25, 0.45, etc…
Vial 3: 0.10, 0.30, 0.50, etc…
Vial 4: 0.15, 0.35, 0.55, etc…
Vial 5: ???

Jerry Campbell
Managing Consultant

D 919-765-8022
jcampbell@ramboll.com

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Wednesday, August 29, 2018 2:25 PM
To: Jerry Campbell <JCampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Sasso, Alan <Sasso.Alan@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Schlosser, Paul <Schlosser.Paul@epa.gov>
Subject: RE: Chloroprene In Vitro model

Jerry, Harvey,
Cc: Alan, Allen

So I've rigged the code and male mouse liver script to read the sample times from the data file and use those for the "injection" decrement. That should make it easy to apply to other experiments (species/tissues). It also has the separate air/medium compartments. "SET10" gives an initial concentration just in the air phase (I used it to check that the simulations fairly match my old BZ model when I try to simulate that).

I now have it plotting for both variable and fixed Km cases, though the fixed Km value was also hand-adjusted for only the male mouse liver data set. That was partly so I could create an acslX plot definition file (.aps, attached) for the comparison.

The revised .csl, male mouse liver .m file, and .aps are attached. Handling the outputs of multiple lengths is clunky, but as much as I'm willing to do right now.

So the issue as I see it is that one needs to know the mass transfer rate between the gas phase and medium in order to correctly interpret the in vitro data. I had assumed that Matt had done those experiments, included the transfer term, what we learned from working with James Bond. The rate will depend on the surface area in the vial and rate of shaking

in the incubator. The rate that I got is clearly too slow to be consistent with the data, but that doesn't mean it's not partially rate-limiting in these experiments. And I don't have a strong intuition for how much it might matter. But the impact will be largest when the rate of metabolism is highest.

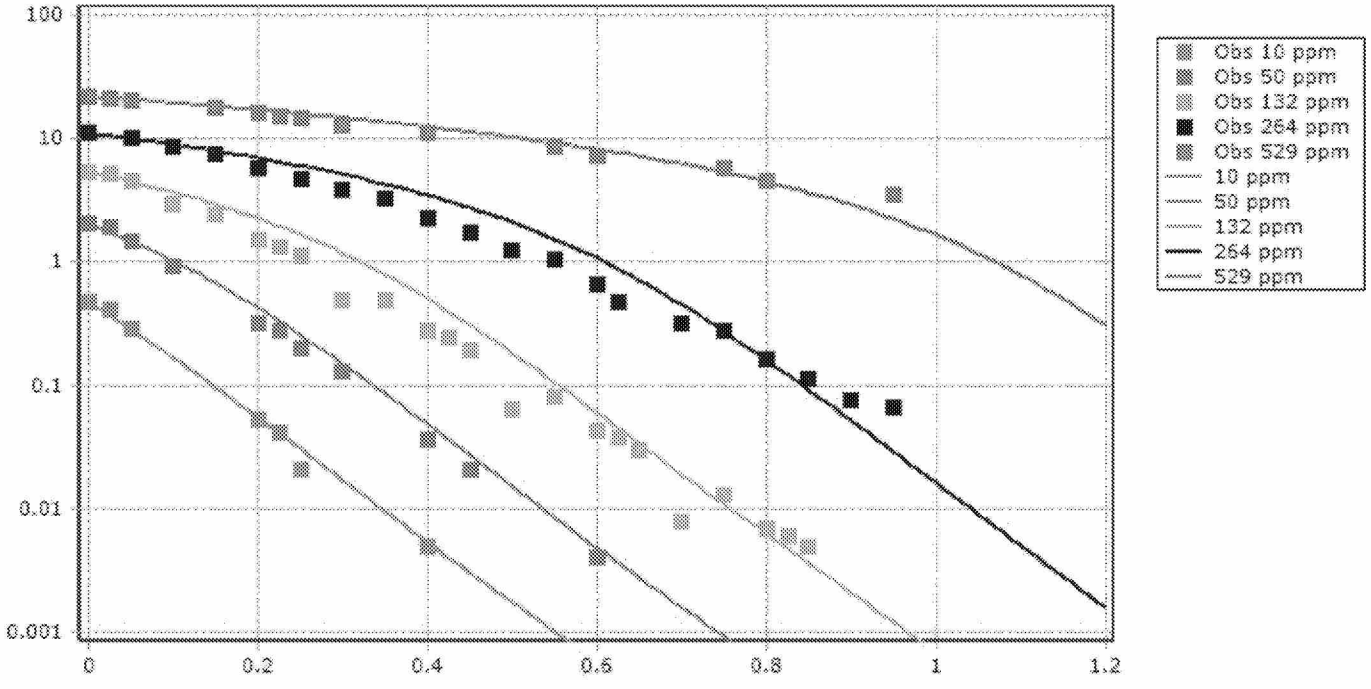
On the other hand, under-counting the sampling (male liver and lung data from Matt) will result in an over-estimate of metabolic rate for those experiments. That will have the largest relative impact when metabolism is slow. At least that just requires an adjustment of the code.

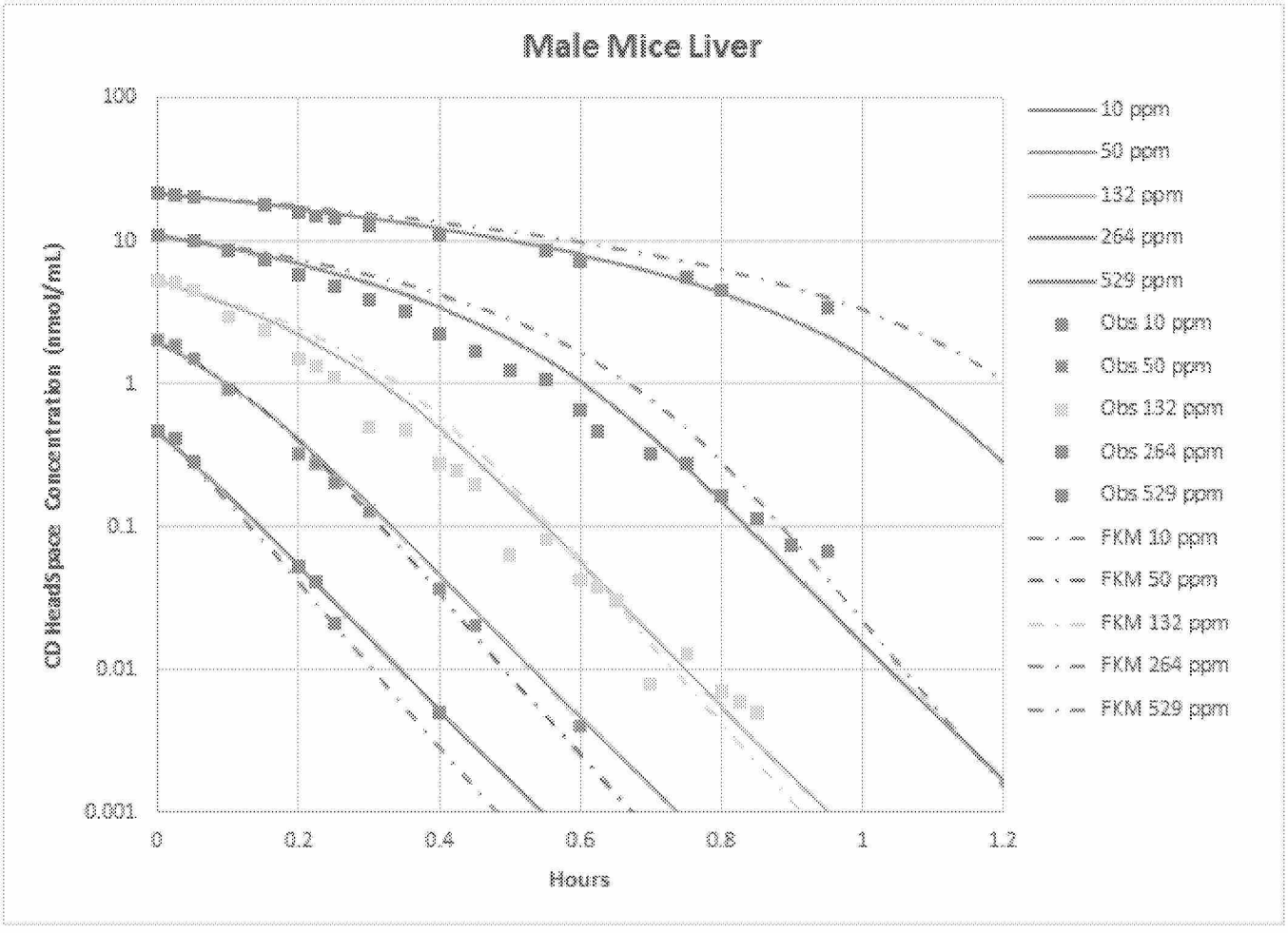
With regards,
-Paul

From: Schlosser, Paul
Sent: Tuesday, August 28, 2018 4:50 PM
To: 'Jerry Campbell' <JCampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

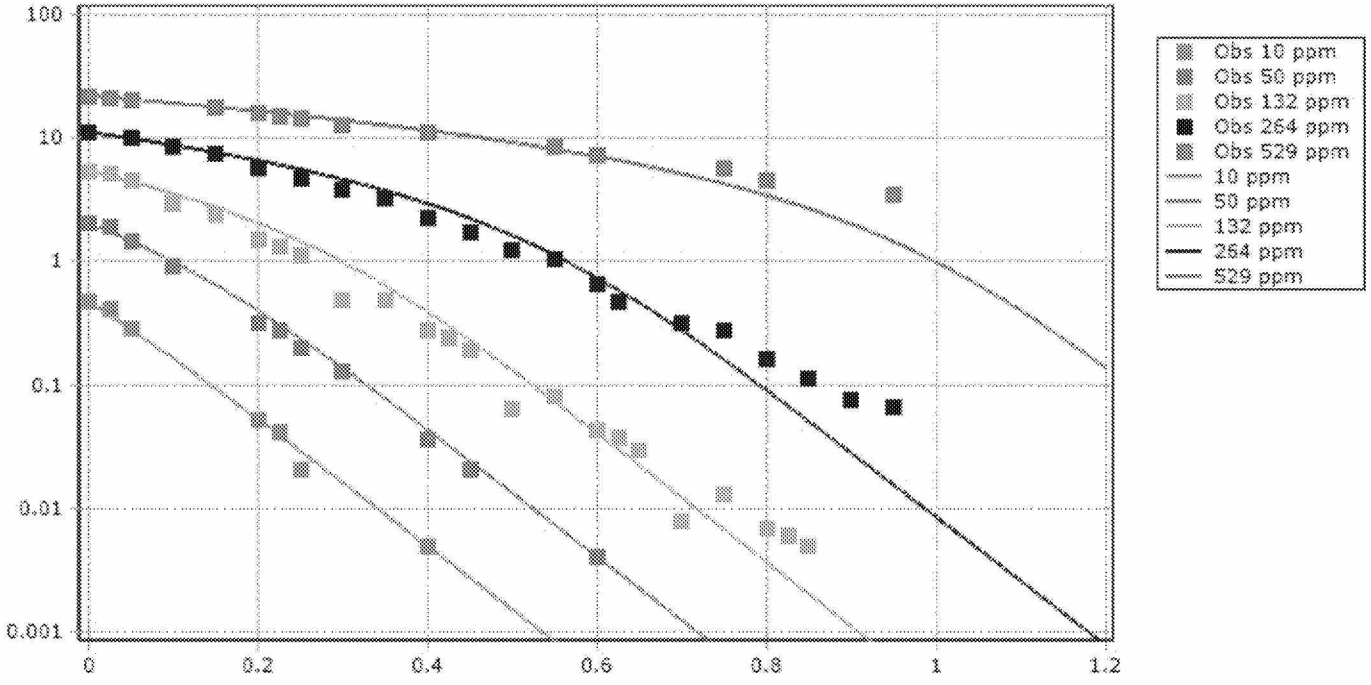
OK, so the first other thing I noticed was that the sampling time (TI) was set to 0.2 h, but clearly samples were taken at a higher frequency. To somewhat quickly get the model to allow for a variation in that, I can't use the procedural, as different sampling intervals changes the length of the output vector, so I can't combine the results in a single array. There's other ways around that, but my cluge was to treat sampling as a continuous loss at rate = VING/TI, where TI is calculated for each data set as TFINAL/NSAMPLE; i.e., the time of the final sample over the number of samples minus the one at time 0.

With the model changed to allow distribution between air and medium (so separate sub-compartments), TI fixed at 0.2 h, but an extremely high mass transfer coefficient (KGL) for air-medium, I get this, compared to the plot (for the Yang parameters) in the spreadsheet that Jerry sent (keep scrolling down):





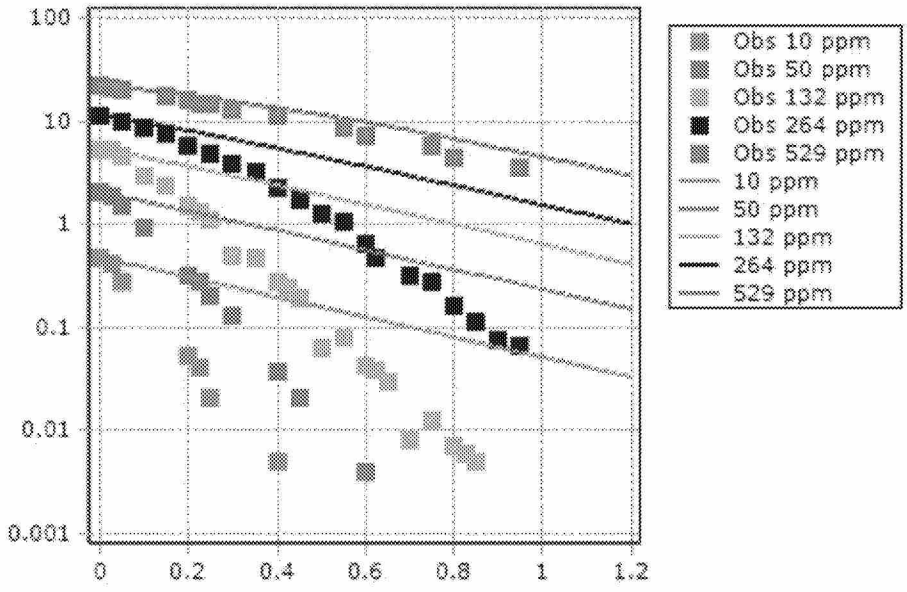
I'd say that's pretty good reproduction! Now, using the variable sampling time (TI), as described above:



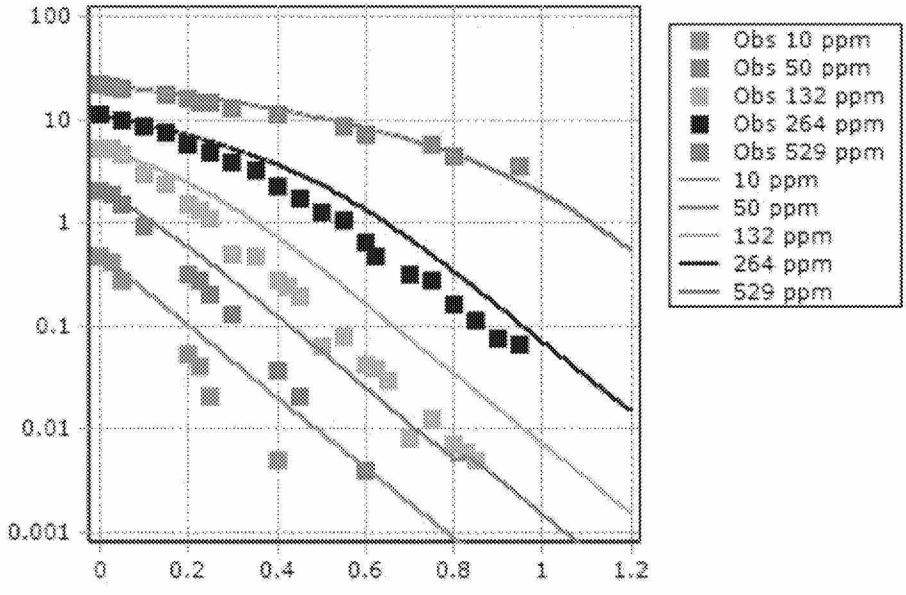
The difference isn't huge, but it's a difference…. For many of the experiments the interval is a fixed 0.2 h, but the male rat and mouse lung, and male rat lung are much more frequent. For the male mouse lung the metabolism is slower, which means the relative impact of this term will be greater.

Ideally the actual sample times should be used, with the scheduled procedural. That's a bit more programming but not terribly difficult. One will just need to deal with the fact that the output from each simulation will be a vector of a different length.

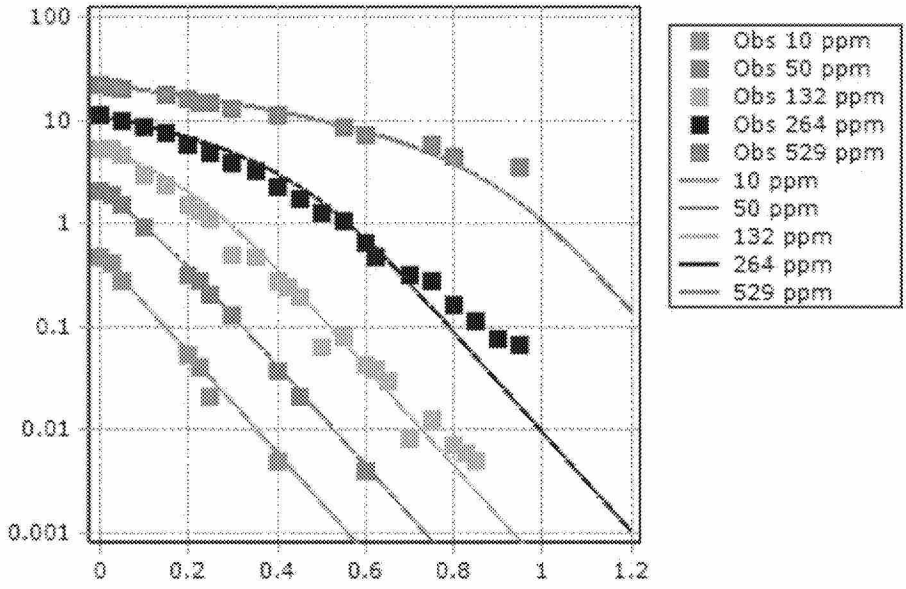
The bigger thing is the gas phase mass transfer. From my '93 benzene paper, the kg = 0.434 ml/min * 60 min/h * 0.001 L/ml = 0.026 L/h. Using that constant, so rate of movement from air to liquid (net) = 0.026*(Ca1 – Cm1/P1), I get:



Really bad, but then there may have been much less mixing in my smaller vials than Matt's, so I increased KGL by 10x, to 0.26:



I then reduced the Km from 1.36 to 0.8 (a bit of trial and error):



Based on this, I'd say that there's a very good chance that the gas-liquid mass transfer is a significant factor, and is likely to impact the estimation of Km (perhaps the goodness of fit of the fixed-Km model). The difficulty is that we need control incubation data to determine the correct value of KGL.

-Paul

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Tuesday, August 28, 2018 11:07 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Harvey Clewell <HClewell@ramboll.com>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Yes, it should be +ARLOSS. I must have hit the wrong key yesterday when I noticed it was missing from the equation.

Jerry Campbell
Managing Consultant

D 919-765-8022
jcampbell@ramboll.com

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, August 28, 2018 8:42 AM
To: Jerry Campbell <JCampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Thanks, Jerry. I've forwarded to Alan who is getting back to his evaluation of the primary model. I'm hoping we can get through the model code evaluation by the end of next week…

Well, I just looked at the .csl and see this:

```
!MASS BALANCE
  CHECK1 = A10 - (A1+A1M+A1I+ ARLUNGVK-ARLOSS)
```

But that should be +ARLOSS?

-Paul

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Monday, August 27, 2018 4:30 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Harvey Clewell <HClewell@ramboll.com>
Subject: Chloroprene In Vitro model

Paul,

I've uploaded a zip folder (INVITROMODEL AND GRAPHS.zip) with the full workspace for the in vitro model and Excel files with the figures. There is a spreadsheet with a list of the m-files and a short description. Let us know if something doesn't work or you have any questions.

Jerry Campbell

Managing Consultant

D 919-765-8022
jcampbell@ramboll.com

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Message

From: HIMMELSTEIN, MATTHEW W [Matthew.W.Himmelstein@dupont.com]
Sent: 8/31/2018 11:37:20 AM
To: Schlosser, Paul [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=121cf759d94e4f08afde0ceb646e711b-Schlosser, Paul]; Jerry Campbell [JCampbell@ramboll.com]
CC: Harvey Clewell [HClewell@ramboll.com]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]
Subject: RE: Chloroprene In Vitro model
Attachments: gerstel pictures.ppt

Paul,

Early in vitro work used a Buker shaker the kind of which we also had at CIIT, and was used for 1,3-butadiene in vitro metabolism as well as for all in vitro blood-to-air gas partitioning work pioneered by Gargas at the WPAFB.

We subsequently switched to a Gerstel head space/incubator/mixer auto sampler attached to the HP GC/MSD (see attached photo) http://www.gerstel.com/en/MPS-Agitator-Incubator-Stirrer.htm. All incubations were conducted with a ~1:10 liquid to air ratio (1 mL in 10 mL vial). My understanding is these facilitates rapid equilibration. Any preincubation time was conducted absent metabolizing protein or NADP. A lot of the initial methods were worked out and published in 2001. Sampling at 12 minute intervals was conducted but as I recall, the start times were staggered to fill in for a more continuous curve using multiple incubation vials.

Hope this helps.

I am out of the office today. Back Tuesday.

Matthew Himmelstein
DuPont Haskell Global Centers
Phone 302 451 4537

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Thursday, August 30, 2018 9:54 AM
To: Jerry Campbell <JCampbell@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: [EXTERNAL] RE: Chloroprene In Vitro model

The other possible check is if experiments were run to check linearity of the initial slope with microsome concentration. I'm pretty sure that if mass transfer resistance is at play, you would see a less-than a doubling of the elimination rate when microsome concentration was doubled.

-Paul

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Subject: RE: Chloroprene In Vitro model

Paul,

There were control experiment data in the 2004 in vitro paper – Figure 3A.

Jerry Campbell
Managing Consultant

D 919-765-8022
jcampbell@ramboll.com

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Subject: FW: Chloroprene In Vitro model

Matt,

As a follow-up, see the email from Jerry below… You can follow the thread below that if you wish!

While the question Jerry asks is if you had gentle mixing going on, the information we really need is on the mass-transfer rate under those conditions. Did you ever run control experiments like the plot below, for another chemical if not CP?

Jerry: note that my incubations were also in a shaker, but I think the amount of surface motion would be dampened considerably in a smaller vial.

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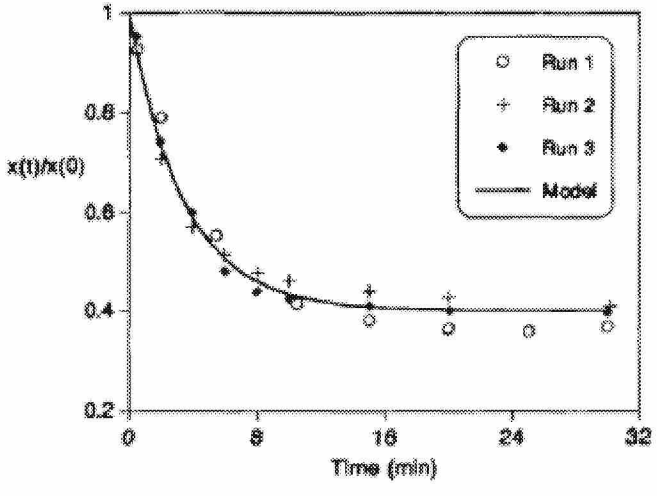

Fig. 3. Partitioning of benzene from liquid phase, into gas phase, in the absence of microsomes under incubation conditions (37°C shaker); $x(t)$ = concentration of benzene in the liquid phase at time = t; $x(0)$ = concentration of benzene in the liquid phase at time = 0. Different initial values, $x(0)$, were used for each run. The model is as depicted in Figure 1 with the rates of biotransformation ($r_1 - r_3$) set to zero.

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Sent: Thursday, August 30, 2018 9:10 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>

Cc: Harvey Clewell <HClewell@ramboll.com>
Subject: RE: Chloroprene In Vitro model

Paul,

Equilibration time question you might want to ask Matt is did they have a shaking sample heater for the headspace vials on their robot? I'm pretty sure they did. The version I had at UGA (was same system sold under another name) had controlled orbital shaking heater that could be set to very slow rotations per min (less than 10/min if I remember correctly) to provide gentle motion. It doesn't say explicitly in the method but it is possible that they used slow rotation to increase surface turnover and decrease liquid equilibration time. We generally used an orbital shaking water bath for non-volatile microsomal metabolism so I wouldn't be surprised if they did include some motion with their analysis too. .

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P.S. If there are data for another chemical where the equilibration rate was measured, those could be used with an adjustment of the PC. But it has to be 100x or more faster than what I measured to give results that are indistinguishable from the model where it's assumed to be instantaneous, and that seems unlikely to me.

Or if not, but someone has the system running for other chemicals, it's only a handful of experiments, no tissues.

-Paul

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Subject: RE: Chloroprene In Vitro model

In essence, there was only one sample scheme (every 0.2 hr or 12 min) but I think it may be more complicated than you have coded. It was an automated system – older version of the combi-pal autosampler. In the more highly sampled incubations (2004 paper in vitro paper), Matt reports that up to 5 vials were used to complete a time-course. So, while there was a mass of sample removed at each time, it wasn't linear throughout the whole run. He does state that samples were taken at 12 min intervals which coincides with the 1 vial system data in the female mouse and rat studies. The question is, can we assume that the 0.2 interval samples in the more highly sampled time-course is from a standardized staggered vial system:

Vial 1: 0, 0.2, 0.4, etc…
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Subject: RE: Chloroprene In Vitro model

Jerry, Harvey,
Cc: Alan, Allen

So I've rigged the code and male mouse liver script to read the sample times from the data file and use those for the "injection" decrement. That should make it easy to apply to other experiments (species/tissues). It also has the separate air/medium compartments. "SET10" gives an initial concentration just in the air phase (I used it to check that the simulations fairly match my old BZ model when I try to simulate that).

I now have it plotting for both variable and fixed Km cases, though the fixed Km value was also hand-adjusted for only the male mouse liver data set. That was partly so I could create an acslX plot definition file (.aps, attached) for the comparison.

The revised .csl, male mouse liver .m file, and .aps are attached. Handling the outputs of multiple lengths is clunky, but as much as I'm willing to do right now.

So the issue as I see it is that one needs to know the mass transfer rate between the gas phase and medium in order to correctly interpret the in vitro data. I had assumed that Matt had done those experiments, included the transfer term, what we learned from working with James Bond. The rate will depend on the surface area in the vial and rate of shaking in the incubator. The rate that I got is clearly too slow to be consistent with the data, but that doesn't mean it's not partially rate-limiting in these experiments. And I don't have a strong intuition for how much it might matter. But the impact will be largest when the rate of metabolism is highest.

On the other hand, under-counting the sampling (male liver and lung data from Matt) will result in an over-estimate of metabolic rate for those experiments. That will have the largest relative impact when metabolism is slow. At least that just requires an adjustment of the code.

With regards,
-Paul

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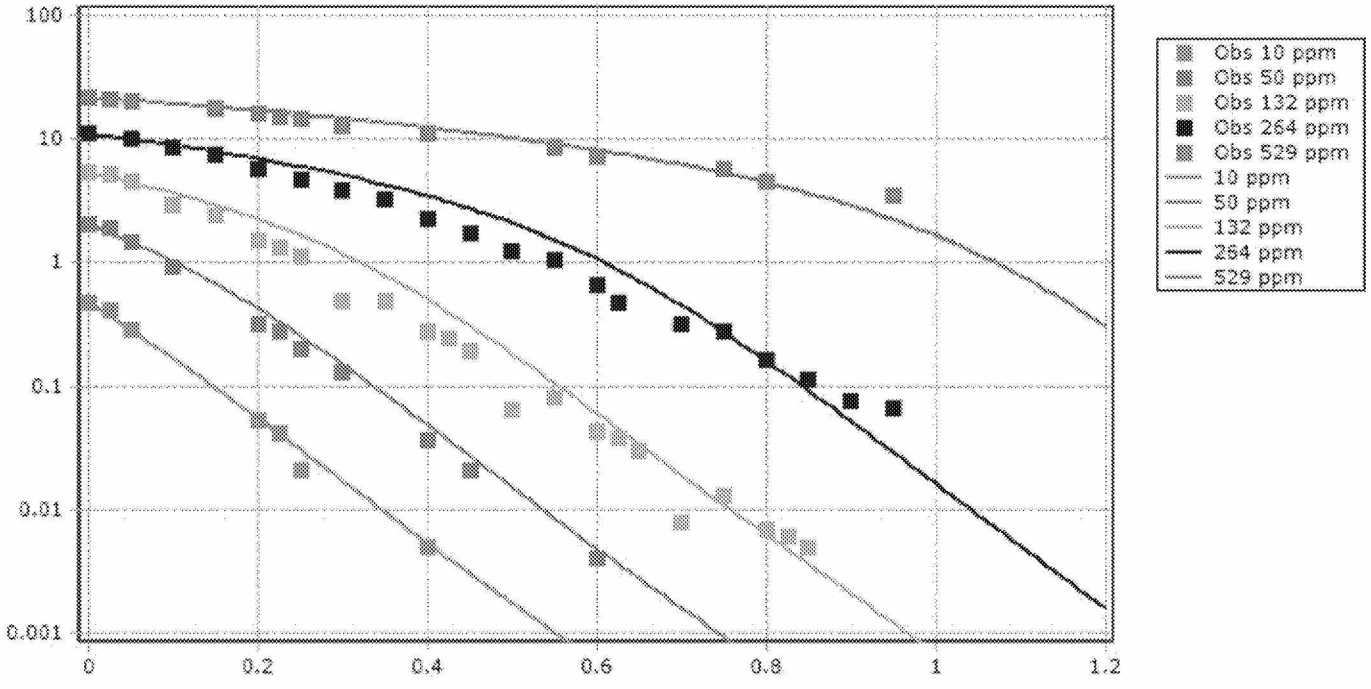
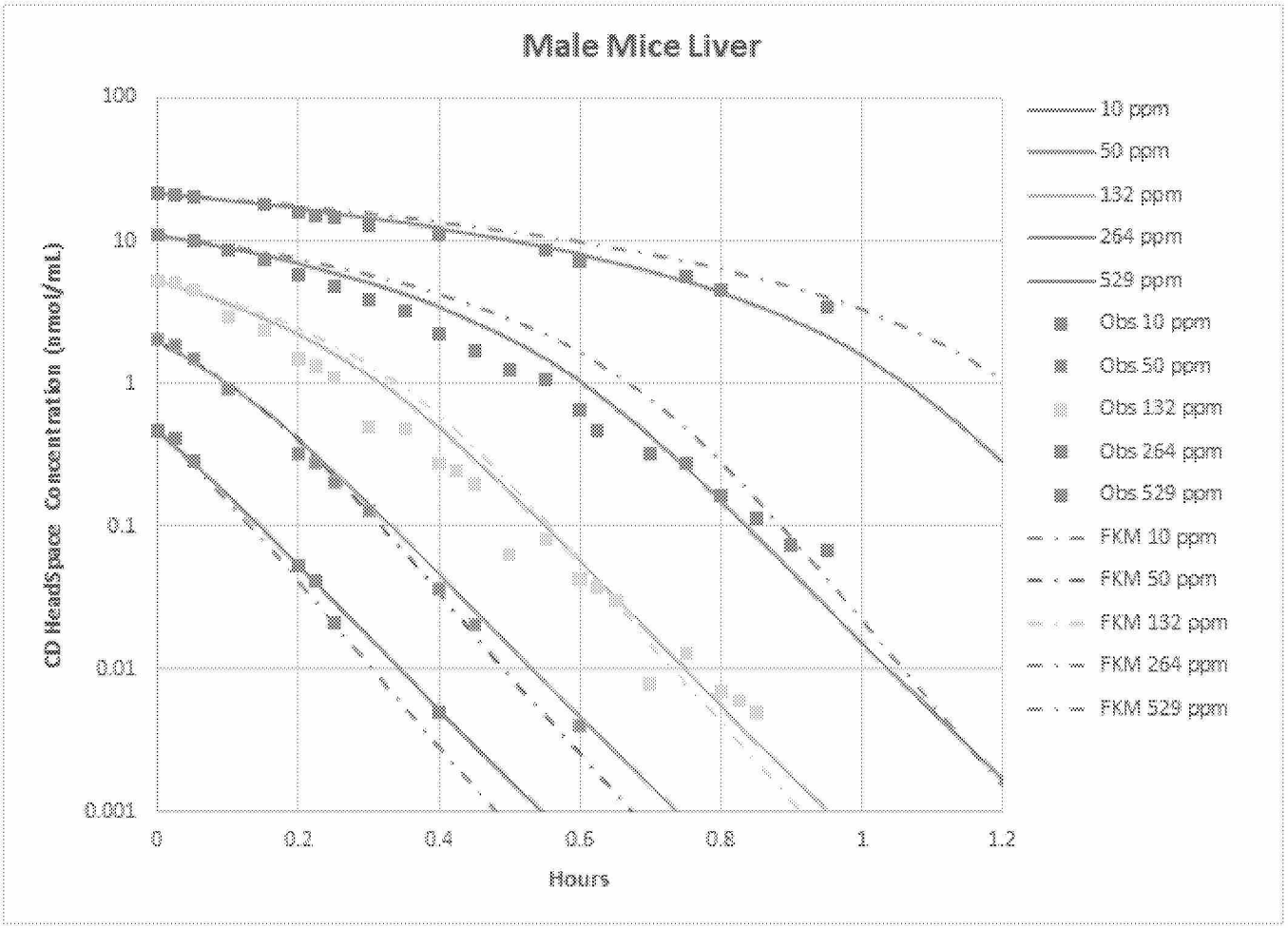
Subject: RE: Chloroprene In Vitro model

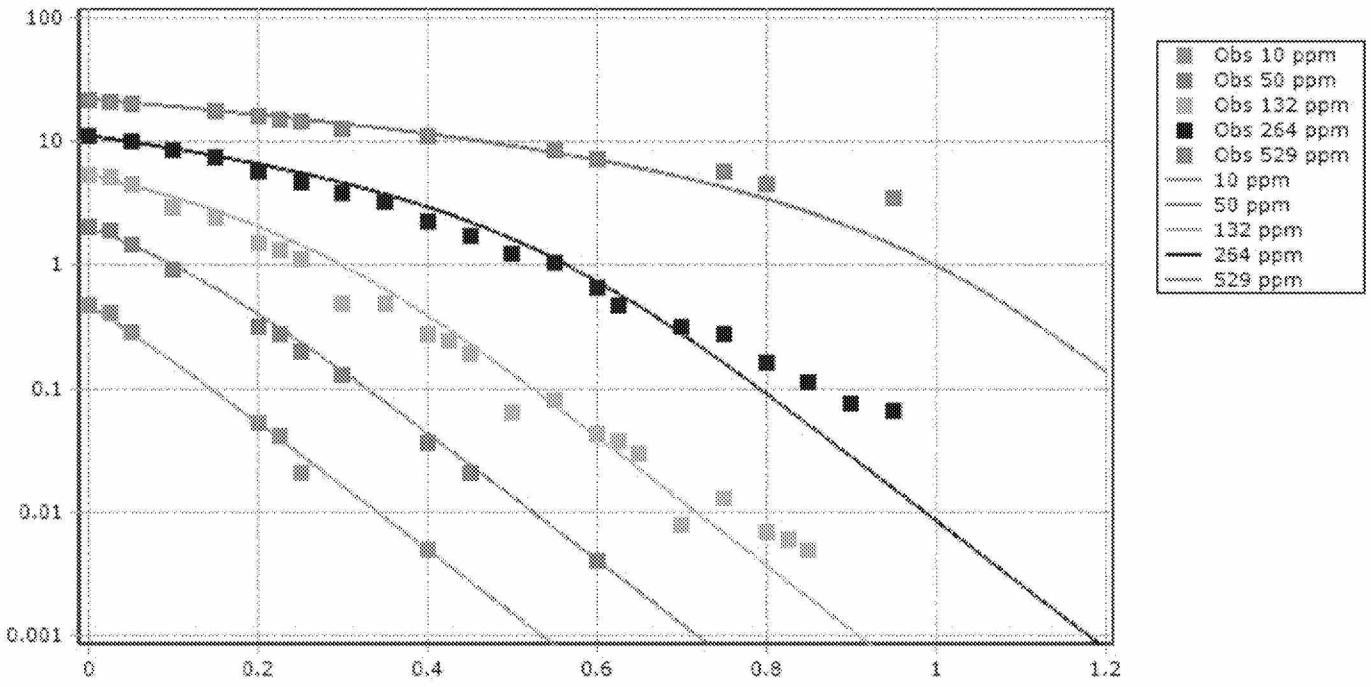
OK, so the first other thing I noticed was that the sampling time (TI) was set to 0.2 h, but clearly samples were taken at a higher frequency. To somewhat quickly get the model to allow for a variation in that, I can't use the procedural, as different sampling intervals changes the length of the output vector, so I can't combine the results in a single array. There's other ways around that, but my cluge was to treat sampling as a continuous loss at rate = VING/TI, where TI is calculated for each data set as TFINAL/NSAMPLE; i.e., the time of the final sample over the number of samples minus the one at time 0.

With the model changed to allow distribution between air and medium (so separate sub-compartments), TI fixed at 0.2 h, but an extremely high mass transfer coefficient (KGL) for air-medium, I get this, compared to the plot (for the Yang parameters) in the spreadsheet that Jerry sent (keep scrolling down):





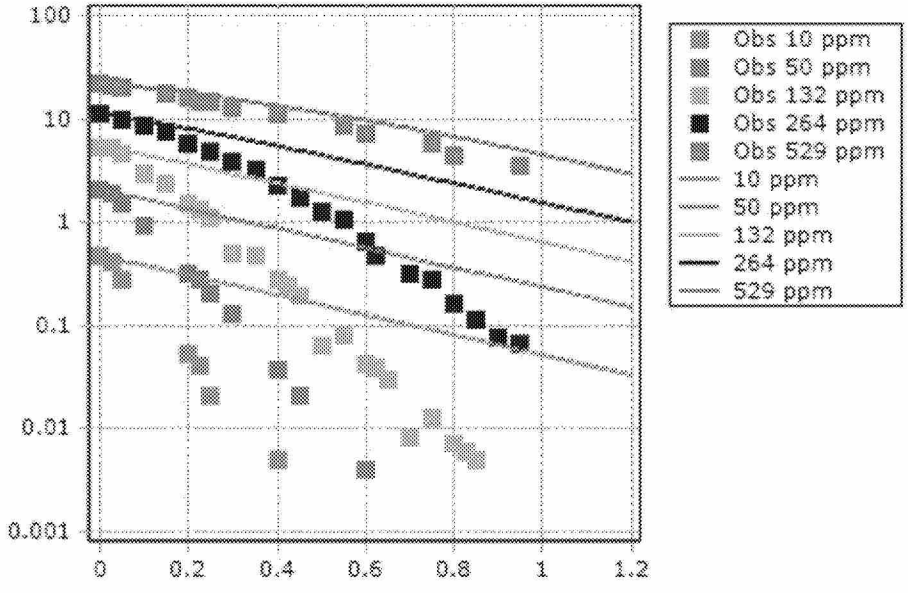
I'd say that's pretty good reproduction! Now, using the variable sampling time (TI), as described above:



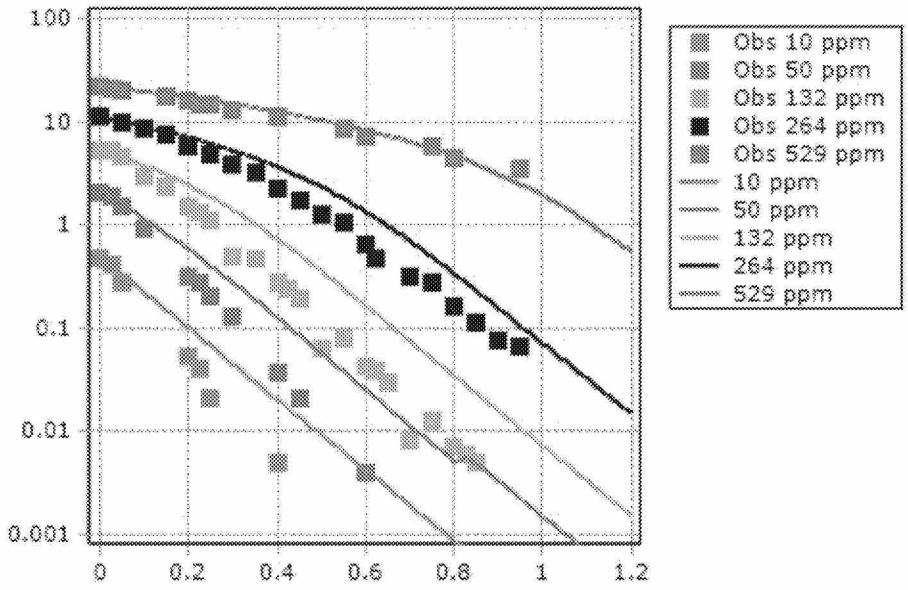
The difference isn't huge, but it's a difference…. For many of the experiments the interval is a fixed 0.2 h, but the male rat and mouse lung, and male rat lung are much more frequent. For the male mouse lung the metabolism is slower, which means the relative impact of this term will be greater.

Ideally the actual sample times should be used, with the scheduled procedural. That's a bit more programming but not terribly difficult. One will just need to deal with the fact that the output from each simulation will be a vector of a different length.

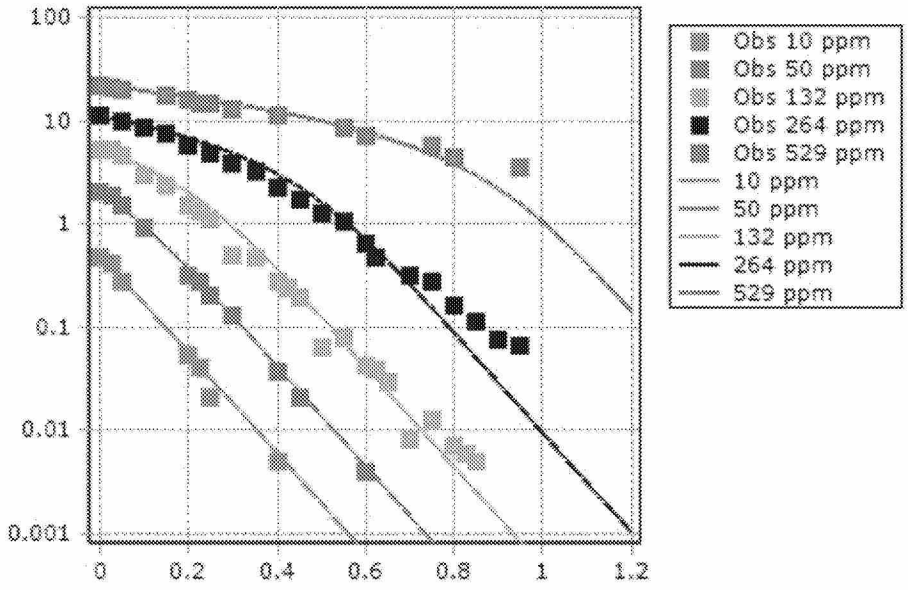
The bigger thing is the gas phase mass transfer. From my '93 benzene paper, the kg = 0.434 ml/min * 60 min/h * 0.001 L/ml = 0.026 L/h. Using that constant, so rate of movement from air to liquid (net) = 0.026*(Ca1 – Cm1/P1), I get:



Really bad, but then there may have been much less mixing in my smaller vials than Matt's, so I increased KGL by 10x, to 0.26:



I then reduced the Km from 1.36 to 0.8 (a bit of trial and error):



Based on this, I'd say that there's a very good chance that the gas-liquid mass transfer is a significant factor, and is likely to impact the estimation of Km (perhaps the goodness of fit of the fixed-Km model). The difficulty is that we need control incubation data to determine the correct value of KGL.

-Paul

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Tuesday, August 28, 2018 11:07 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>

Cc: Harvey Clewell <HClewell@ramboll.com>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Yes, it should be +ARLOSS. I must have hit the wrong key yesterday when I noticed it was missing from the equation.

Jerry Campbell
Managing Consultant

D 919-765-8022
jcampbell@ramboll.com

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, August 28, 2018 8:42 AM
To: Jerry Campbell <JCampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Thanks, Jerry. I've forwarded to Alan who is getting back to his evaluation of the primary model. I'm hoping we can get through the model code evaluation by the end of next week…

Well, I just looked at the .csl and see this:

```
!MASS BALANCE
  CHECK1 = A10 - (A1+A1M+A1I+ ARLUNGVK-ARLOSS)
```

But that should be +ARLOSS?

-Paul

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Monday, August 27, 2018 4:30 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Harvey Clewell <HClewell@ramboll.com>
Subject: Chloroprene In Vitro model

Paul,

I've uploaded a zip folder (INVITROMODEL AND GRAPHS.zip) with the full workspace for the in vitro model and Excel files with the figures. There is a spreadsheet with a list of the m-files and a short description. Let us know if something doesn't work or you have any questions.

Jerry Campbell

Managing Consultant

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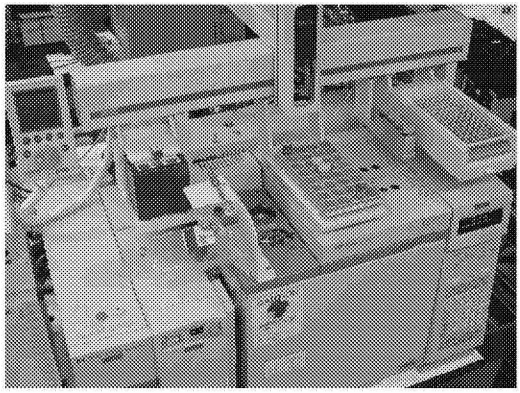
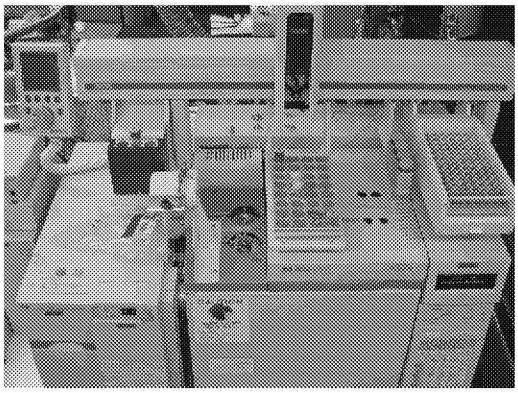
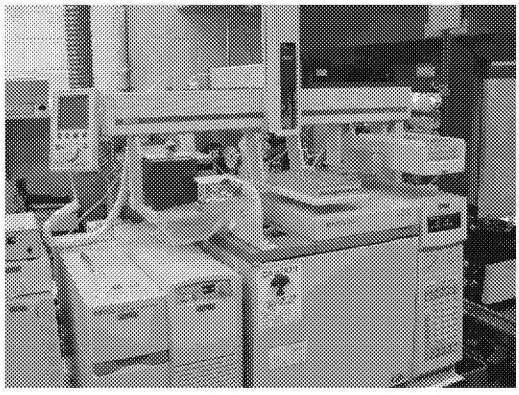
6 Davis Drive
Suite 139
PO Box 13441
Research Triangle Park
NC 27709
USA
www.ramboll.com







Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 8/30/2018 1:54:03 PM
To: Jerry Campbell [JCampbell@ramboll.com]; HIMMELSTEIN, MATTHEW W [Matthew.W.Himmelstein@dupont.com]
CC: Harvey Clewell [HClewell@ramboll.com]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]
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The other possible check is if experiments were run to check linearity of the initial slope with microsome concentration. I'm pretty sure that if mass transfer resistance is at play, you would see a less-than a doubling of the elimination rate when microsome concentration was doubled.

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Paul,

There were control experiment data in the 2004 in vitro paper – Figure 3A.

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Matt,

As a follow-up, see the email from Jerry below… You can follow the thread below that if you wish!

While the question Jerry asks is if you had gentle mixing going on, the information we really need is on the mass-transfer rate under those conditions. Did you ever run control experiments like the plot below, for another chemical if not CP?

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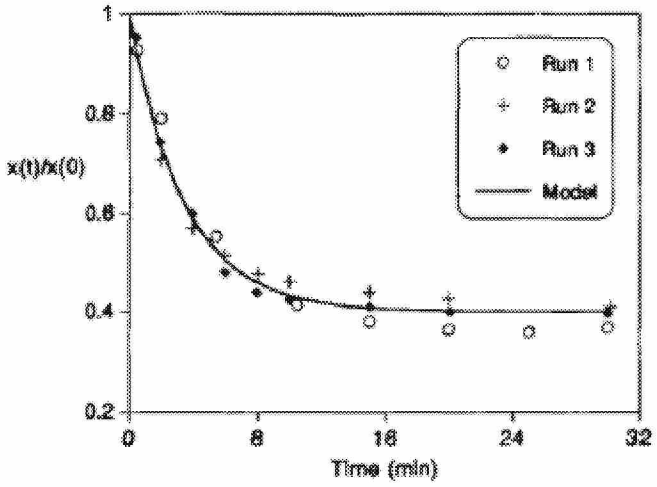

Fig. 3. Partitioning of benzene from liquid phase, into gas phase, in the absence of microsomes under incubation conditions (37°C shaker); $x(t)$ = concentration of benzene in the liquid phase at time = t; $x(0)$ = concentration of benzene in the liquid phase at time = 0. Different initial values, $x(0)$, were used for each run. The model is as depicted in Figure 1 with the rates of biotransformation ($r_1 - r_3$) set to zero.

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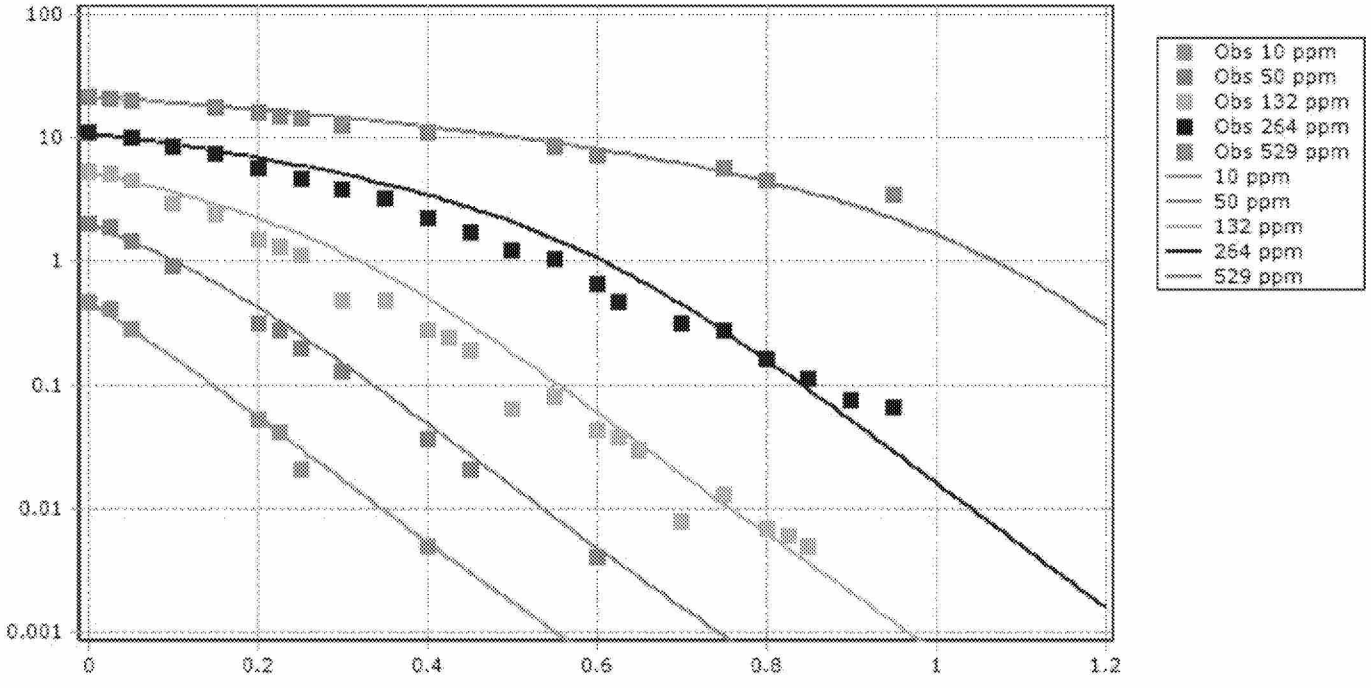
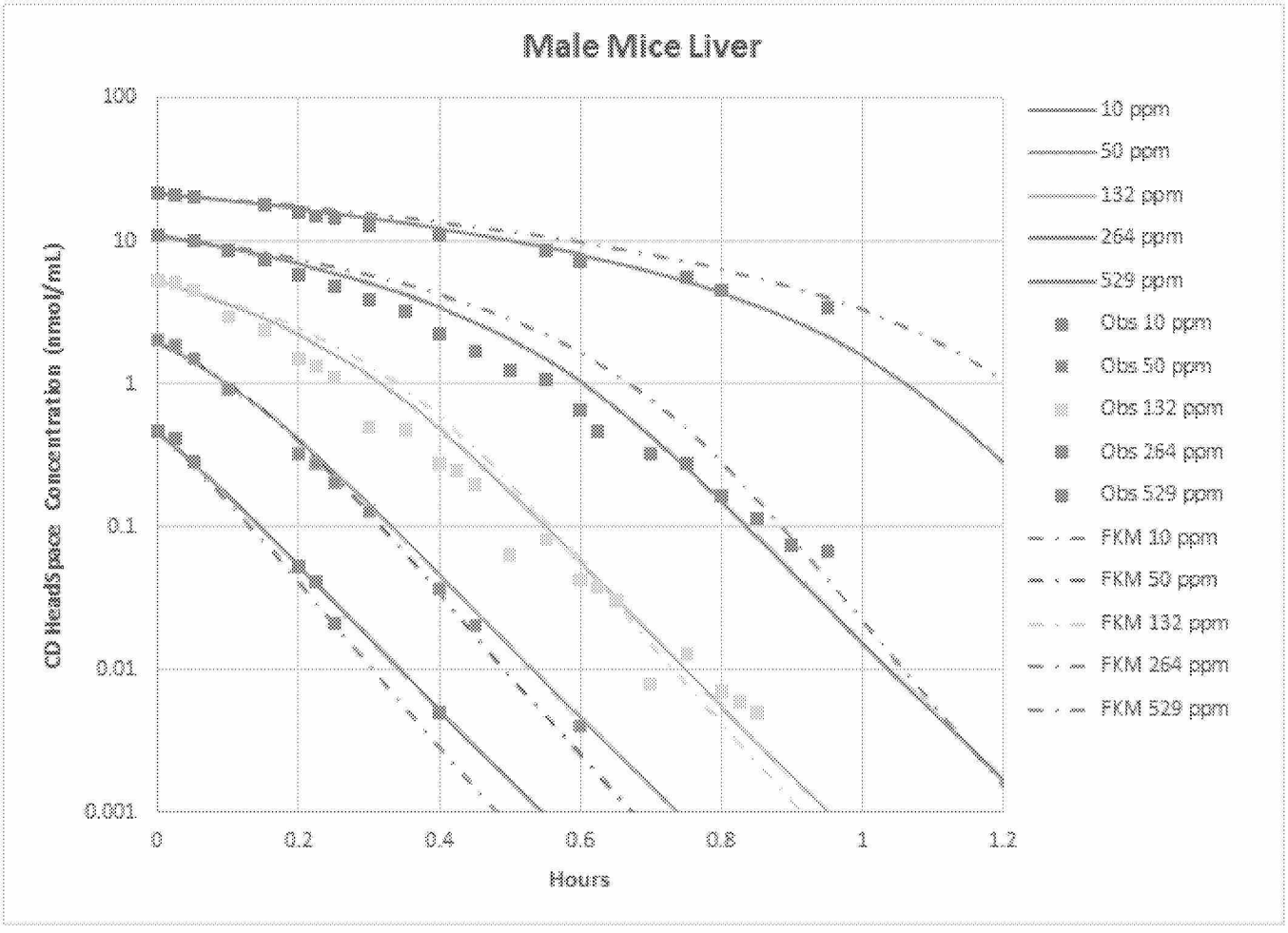
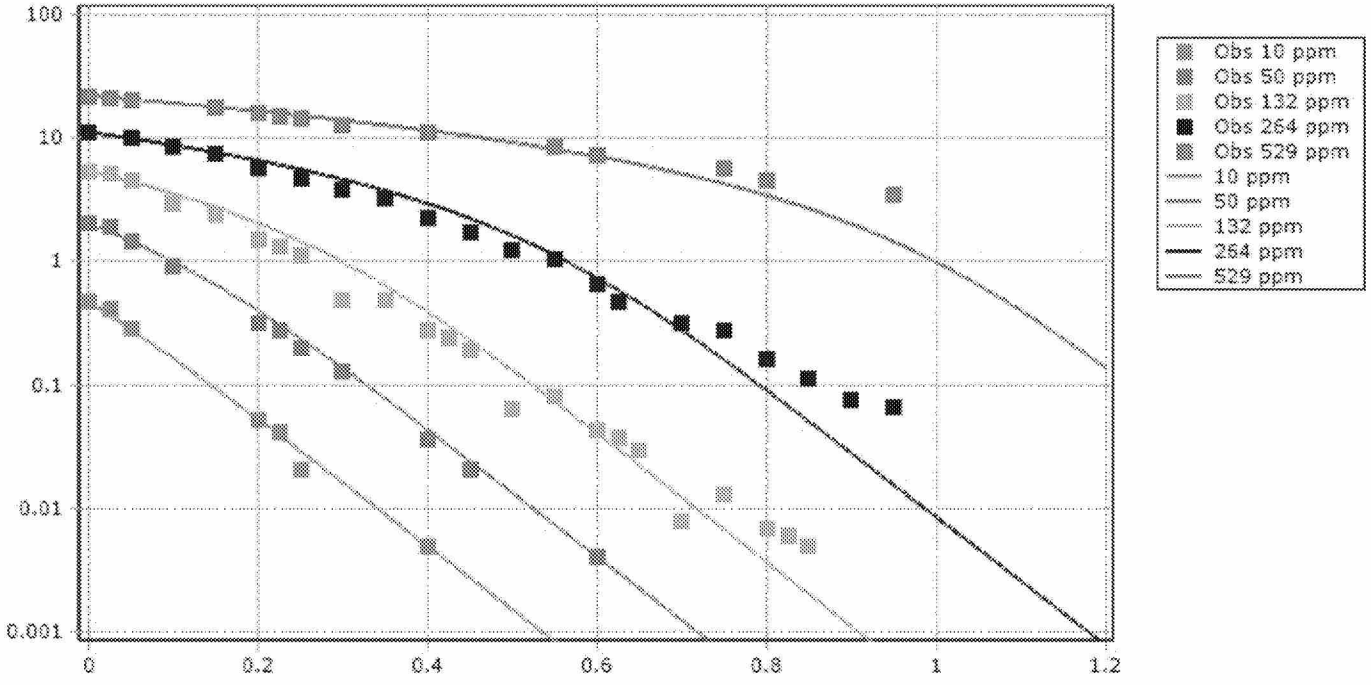
With the model changed to allow distribution between air and medium (so separate sub-compartments), TI fixed at 0.2 h, but an extremely high mass transfer coefficient (KGL) for air-medium, I get this, compared to the plot (for the Yang parameters) in the spreadsheet that Jerry sent (keep scrolling down):





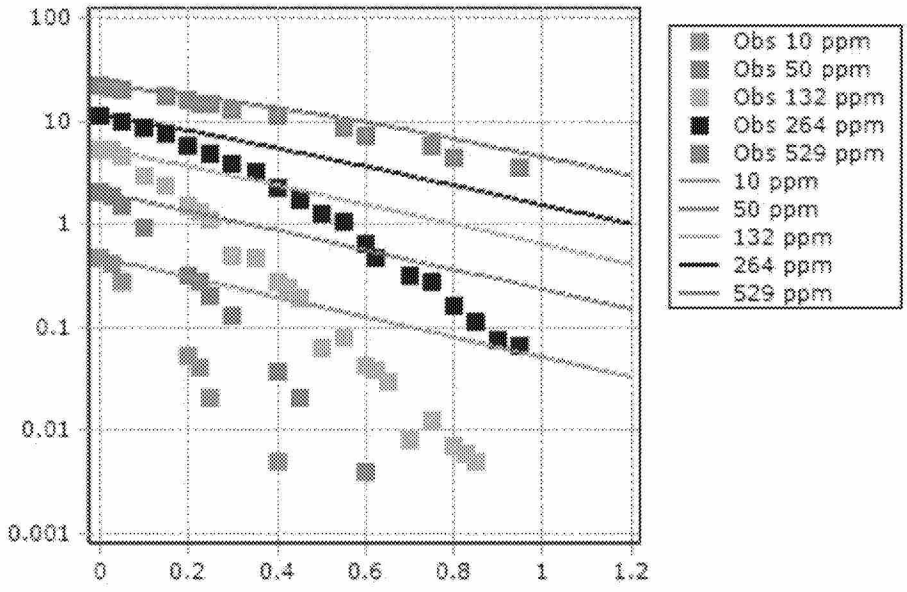
I'd say that's pretty good reproduction! Now, using the variable sampling time (TI), as described above:



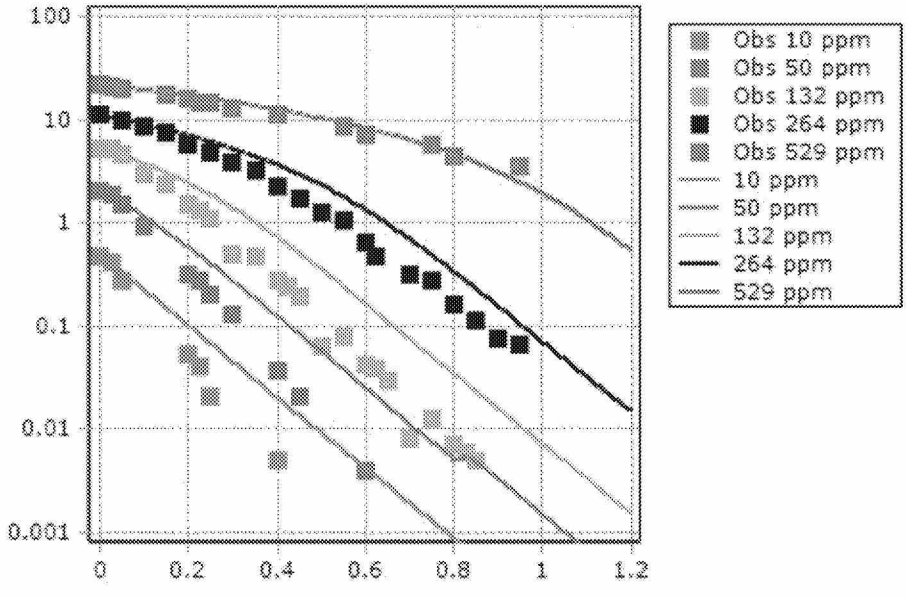
The difference isn't huge, but it's a difference…. For many of the experiments the interval is a fixed 0.2 h, but the male rat and mouse lung, and male rat lung are much more frequent. For the male mouse lung the metabolism is slower, which means the relative impact of this term will be greater.

Ideally the actual sample times should be used, with the scheduled procedural. That's a bit more programming but not terribly difficult. One will just need to deal with the fact that the output from each simulation will be a vector of a different length.

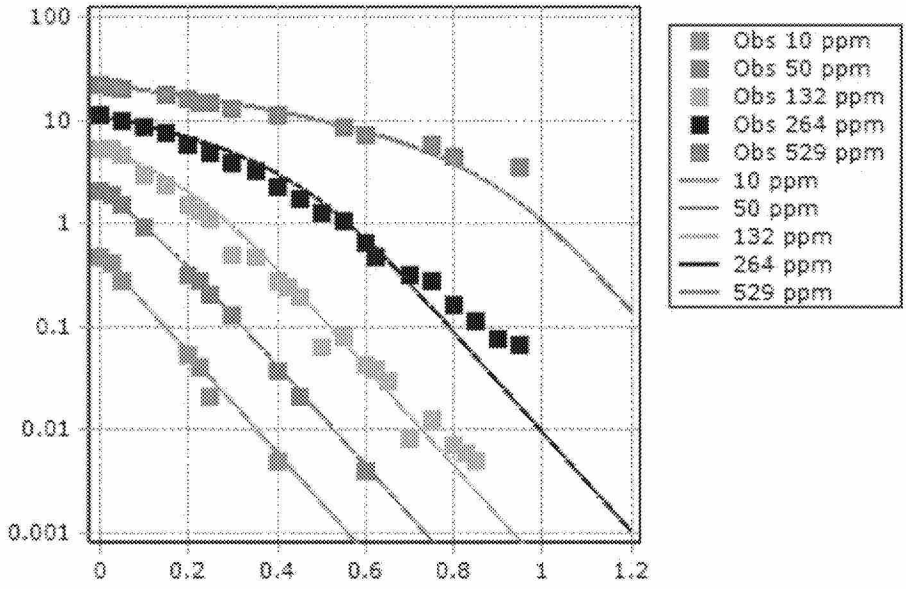
The bigger thing is the gas phase mass transfer. From my '93 benzene paper, the kg = 0.434 ml/min * 60 min/h * 0.001 L/ml = 0.026 L/h. Using that constant, so rate of movement from air to liquid (net) = 0.026*(Ca1 – Cm1/P1), I get:



Really bad, but then there may have been much less mixing in my smaller vials than Matt's, so I increased KGL by 10x, to 0.26:



I then reduced the Km from 1.36 to 0.8 (a bit of trial and error):



Based on this, I'd say that there's a very good chance that the gas-liquid mass transfer is a significant factor, and is likely to impact the estimation of Km (perhaps the goodness of fit of the fixed-Km model). The difficulty is that we need control incubation data to determine the correct value of KGL.

-Paul

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Tuesday, August 28, 2018 11:07 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Harvey Clewell <HClewell@ramboll.com>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Yes, it should be +ARLOSS. I must have hit the wrong key yesterday when I noticed it was missing from the equation.

Jerry Campbell
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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, August 28, 2018 8:42 AM
To: Jerry Campbell <JCampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Thanks, Jerry. I've forwarded to Alan who is getting back to his evaluation of the primary model. I'm hoping we can get through the model code evaluation by the end of next week…

Well, I just looked at the .csl and see this:

```
!MASS BALANCE
  CHECK1 = A10 - (A1+A1M+A1I+ ARLUNGVK-ARLOSS)
```

But that should be +ARLOSS?

-Paul

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Monday, August 27, 2018 4:30 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Harvey Clewell <HClewell@ramboll.com>
Subject: Chloroprene In Vitro model

Paul,

I've uploaded a zip folder (INVITROMODEL AND GRAPHS.zip) with the full workspace for the in vitro model and Excel files with the figures. There is a spreadsheet with a list of the m-files and a short description. Let us know if something doesn't work or you have any questions.

Jerry Campbell

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Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 6/28/2019 5:47:55 PM
To: Harvey Clewell [HClewell@ramboll.com]
CC: Jerry Campbell [JCampbell@ramboll.com]; Michael Dzierlenga [MDZIERLENGA@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; mandersen [andersenme@aol.com]; Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Morozov, Viktor [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=03cc9abb639c453fabc2bbb3e4617228-Morozov, Viktor]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Kapraun, Dustin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3a53c151b92a472fbfb295ed5df982a7-Kapraun, Du]; Sonja Sax [SSax@ramboll.com]; Ken Mundt [kenneth.mundt@cardno.com]; Miyoung Yoon [yoon.m.work@gmail.com]; Kenyon, Elaina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0395d5b93f214c8ca49066f498f7d5c9-Kenyon, Elaina]; White, Paul [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4e179825823c44ebbb07a9704e1e5d16-White, Paul]
Subject: RE: chloroprene -- human lung
Attachments: Botto_94.pdf

Yes, I see that now. I'd been looking in the methods section, then went to the prior paper which has results on liver (larger n). And this other paper (attached) that they cite also only had one lung sample, but at least shows the variation from repeating the microsome prep 3 times (Table 2), which is modest.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Friday, June 28, 2019 10:10 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <andersenme@aol.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <yoon.m.work@gmail.com>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>; White, Paul <White.Paul@epa.gov>
Subject: RE: chloroprene -- human lung

Hi Paul

I checked with Miyoung and the human values in Table 3 are based on Viera et al. (1998) Table 1, which provides data from a single adult subject.

With kind regards
Harvey Clewell

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Sent: Thursday, June 27, 2019 2:14 PM
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Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <andersenme@aol.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <yoon.m.work@gmail.com>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>; White, Paul <White.Paul@epa.gov>
Subject: RE: chloroprene -- human lung

Thanks, Harvey.

The pool size could be bigger, but it is good support that the Lorenz data don't under-estimate human lung activity. … On the other hand, how could I forget?! There is this paper from Miyoung, attached. The ratio shown in table 3 is 0.9%, or 0.009. It is citing

Vieira, I., Pasanen, M., Raunio, H., and Cresteil, T. 1998. Expression of CYP2E1 in human lung and kidney during development and in full-term placenta: A differential methylation of the gene is involved in the regulation process. Pharmacol. Toxicol. 83:183–187, which in turn cites a 1996 article describing the initial tissue collection. Both are also attached, sorry about the rotation of the 1st page of the '98 paper, it's how it is on HERO.

From the '96 paper: Adult liver samples were obtained from donors for kidney transplantation. Donors had no severe chronic pathology and had generally died from a traffic accident. They
had no re-peated drug consumption. No information was available regarding their smoking and drinking habits.

Some plots in the '96 paper indicate 14 donors, others 10 donors.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Thursday, June 27, 2019 10:40 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <andersenme@aol.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <yoon.m.work@gmail.com>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>; White, Paul <White.Paul@epa.gov>
Subject: RE: chloroprene -- human lung

Hi Paul

This paper provides relative expression ratios across tissues in the human for a variety of cyps.

From Table 1, the ratio of lung/liver expression for 2e1 is 0.0173/53.8 = 0.00032

Adding 2F1 in the lung (which was not detected in the liver), it becomes (0.0173 + 0.0128)/53.8 = 0.00056

That's about a factor of 3 lower than the lung/liver activity ratio from Lorenz (A1 = 0.00143)

This supports the use of A1 derived from Lorenz to estimate human lung metabolism for 2e1 substrates like chloroprene or methylene chloride as a conservative approach.

With kind regards
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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, June 25, 2019 5:26 PM
To: Jerry Campbell <JCampbell@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <andersenme@aol.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <yoon.m.work@gmail.com>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>; White, Paul <White.Paul@epa.gov>
Subject: chloroprene -- human lung

Harvey, all,

The Lorenz et al. (1984) paper from which the 'A1' for lung:liver metabolism is calculated use 7-ethoxycoumarin as a substrate, which is not a pure 2E1 substrate, but also metabolized by human 1A2, which would not be relevant for CP, and some others.

https://www.ncbi.nlm.nih.gov/pubmed/8573198
https://www.ncbi.nlm.nih.gov/pubmed/16719387

I didn't look thoroughly, but didn't see that Lorenz gave the concentration of 7-EC they used (the 1st reference above indicates that at high concentrations it's more 2E1-specific), and the paper they cite for the method is a review paper. I stopped at that point.

Also, while Lorenz had data on 13 separate human subjects for liver metabolism and 10 for lung (all non-smokers!), they were all having biopsies or surgery for a reason.

No human data set is ideal, but do you know of a 3rd data set for human lung vs. liver activity we could consider, to triangulate the thing, as it were?

-Paul

Pergamon

Biochemical Pharmacology, Vol. 48, No. 6, pp. 1095–1103, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)00286-X

TISSUE-SPECIFIC EXPRESSION AND METHYLATION OF THE HUMAN *CYP2E1* GENE

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(*Received* 8 *December* 1993; *accepted* 7 *June* 1994)

Abstract—The level and number of CYP2E1 gene transcripts were investigated by northern blot analysis in various human adult tissues including liver, lung, placenta, skin and neurinoma. Three transcripts of 1.8, 2.6 and 4 Kb were expressed in a tissue-specific manner. The origin of the various transcripts was studied and showed that both 4 and 2.6 Kb mRNAs contained sequences from the 3′ non-translated region of the gene and that the 4 Kb also contained region localized in the 5′ non-translated region. Furthermore, it clearly appeared that a catalytically active CYP2E1 enzyme (as proved by NDMA demethylase activity) was only detected in tissues expressing the 1.8 Kb. The human CYP2E1 was also identified through immunohistochemical techniques. Finally, we observed a relation between the hypomethylation of the human *CYP2E1* gene and the hypoexpression of the corresponding protein.

Key words: gene expression; DNA methylation; cytochrome P450; CYP2E1; *N*-nitrosodimethylamine metabolism, immunochemical evidence

Cytochrome P450s belong to a multigene family of enzymes [1]. These proteins are mostly expressed in the liver and to a lesser extent in numerous extrahepatic tissues including lung [2], small intestine [3], lymphocytes [4], bone marrow [5], kidney [6] and nasal mucosa [6, 7]. Some cytochrome P450s are constitutively expressed in untreated animals and some can be induced by treatment with specific compounds [8]. Cytochrome P450s are involved in the metabolism of exogenous compounds (such as drugs, carcinogens and pesticides) as well as endogenous substances (such as fatty acids, prostaglandins, steroids and ketone bodies produced during fasting). They can either convert xenobiotics to inert polar metabolites or bioactivate them to reactive species. Some cytochrome P450s are modified by physiopathological conditions such as fasting, diabetes, age, sex, stress or by exposure to various chemical compounds (environmental pollutents, drugs etc.).

One of the most widely studied cytochrome P450s is the ethanol-inducible CYP2E1. The regulation of expression of this cytochrome is one of the most complex. Indeed, it involves regulation: (1) at the level of transcription at birth (with an associated demethylation [9, 10]) (2) by mRNA stabilization in the diabetic state and by fasting; (3) by increased translation of existing mRNA; and (4) by inhibition of protein degradation [11]. CYP2E1 is crucial since it is implicated in the biotransformation of chemicals which have toxic effects in humans (styrene, vinyl chloride [12, 13]) and in the bioactivation of precarcinogens (benzene, *N*-nitrosodimethylamine etc. [14]). These reactions are generally dependent on the CYP2E1 protein level, which has been found to be elevated in: (1) people suffering from alcoholism [15]; (2) patients undergoing therapy with isoniazid [16]; (3) diabetic and especially those who do not respond to insulin [4].

The aim of the present study was to analyse the variations in expression of the *CYP2E1* gene (mRNA and protein) in various human adult tissues using enzymatic assay (NDMA§ demethylation), immunohistochemical experiments, northern hybridization and to explain them at least in part (characterization of the different transcripts and gene methylation).

MATERIALS AND METHODS

Reagents. [α-^{32}P]dATP and [γ-^{32}P]ATP were obtained from Amersham (Amersham, U.K.). NDMA was purchased from Sigma (Saint Quentin Fallavier, France). Antibodies conjugated to alkaline phosphatase or horse radish peroxidase were from Biosys (Compiegne, France). The rabbit anti-rat CYP2E1 IgG was kindly provided by F.J. Gonzalez [17]. Restriction enzymes Hpa II, Msp I, Hind III and BamH I were obtained from Boehringer (Mannheim, F.R.G.). Moviol was from Calbiochem (Frankfurt, F.R.G.). All other reagents were of the highest quality available.

Human samples. A variety of human adult tissues were studied including: liver, placenta, skin, lung and neurinoma. The livers were obtained under strict ethical conditions from organ donors. The placenta were from full term pregnancies after

* Corresponding author.

§ Abbreviations: IgG, immunoglobulin; NDMA, *N*-nitrosodimethylamine; NGS, normal goat serum; PCR, polymerase chain reaction; SSC, standard saline citrate

Table 1. CYP2E1 oligonucleotides used in this study

CYP2E1 probe	Length (bp)	Localization	Ref.	Sequence (5′–3′)
B	40	11,503–11,542	34	Complementary to an intronic segment in the 3′ flanking region localized after the AATAAA site: CAA GAT CAT GCC ACT GCA CTC CAT CCT GGT CAA CAA GAG C
C	20	−819–−800	34	Identical to an intronic segment localized in the 5′ flanking region: TAT TGT GCG CCG GGA TCA AC
D	20	11,335–11,354	34	Identical to an intronic segment in the 3′ flanking region localized before the AATAAA site: CTG ATT CCT TTC TTT GCA TA
E	20	−819–−800	34	Complementary to an intronic sequence localized in the 5′ flanking region: GTT GAT CCC GGC GCA CAA TA
F	40	77–116	34	Complementary to a region in the first exon: TGC ACC TGC CTC CAC ATG GAC ACC AGC AGG AGG AAG GCC G
G	20	4801–4820	34	Complementary to a region in the fourth exon: AGG ATG TCG GCT ATG ACG TT
H	20	5101–5120	34	Complementary to a region in the fourth intron: CAC ATC CTG ACG TTA GGA AA
I	20	11,944–11,963	34	Complementary to an intronic segment in the 3′ flanking region localized after the AATAAA site: ACT CCC TTT CGT ATA TAC AT
J	20	828–847	10	Complementary to regions localized in exons 5 and 6: AAT GGA GAA GGA AAA GCA CA
K	17	971–987	10	Complementary to regions localized in exons 6 and 7: GAG CTT CTC TTC GAT CT

normal deliveries. The mothers were healthy, drug-free and non smokers. Placenta were washed with ice-cold sterile physiological solution to eliminate blood. Left acoustic neurinoma and lung biopsies were obtained from surgical resection of tumours. Skin was obtained from women after breast plastic surgery. All tissues were kept frozen within half an hour after removal and stored at −80° until use.

Cloning of the human P450A cDNA probe. Cytochrome P450A cDNA was isolated from a human adult liver cDNA library [18], cloned into the Pst I site of the pBR 322. Screening was done with the R17 (*CYP2B2*) [19] probe under non-stringent conditions. A clone (P450A) was selected according to its highest hybridization with this probe and after sequencing (following the method of Sanger *et al.* [20]) appeared to be part of the *CYP2E1* cDNA (position 749 to 1623 [10]).

Preparation of radioactive probes. ^{32}P-labelled single-strand cDNA probes, GAPDH (glyceraldehyde-3-phosphate deshydrogenase) [21] and probe A, were prepared by incorporation of [α-^{32}P]-dATP by primer extension [22] using the Klenow polymerase. The oligonucleotides used for hybridization analysis are shown in Table 1. Oligonucleotides (6 pmol) were 5′ labelled with γ-^{32}P by incubating with T4 polynucleotide kinase [22].

RNA extraction, electrophoresis and blot analysis. Total cellular RNA was isolated from various human tissues following the procedure described by Sambrook *et al.* [22]. Total RNA (10 μg) was fractionated on a 12.3 M formaldehyde–1.4% agarose gel, transferred to nitrocellulose membrane (0.2 μm, Schleicher and Schuell) as described previously [23] and baked at 80° for 2 hr. Total RNA extracted from normal human liver 3 was run as control in each analysis. The blots were prehybridized 2 hr at 68° in 6 × SSC (20 × SSC is: 3 M NaCl, 0.3 M citric acid; pH = 7.0), 10% dextran sulphate, 5 × Denhardt's (100 × Denhardt's solution is: 2% polyvinyl pyrrolidone, 2% Ficoll, 2% BSA in 3 × SSC), and hybridized at 45° in the same solution containing 10^6 cpm/mL of the labelled oligonucleotide probes. The filters were also prehybridized in 5 × SSC, 10% dextran sulphate, 5 × Denhardt's, 50% freshly deionized formamide and hybridized at 45° in the same solution containing 10^5 cpm/mL of the labelled A or GAPDH probes. The filters were then washed three times at 45° in either 6 × SSC or 2 × SSC. The blots were exposed at −80° with an intensifying screen. The autoradiogram intensities of the 1.8 Kb mRNA band were estimated by scan densitometry and normalized to GAPDH; this was noted REL for Relative Expression Level:REL = Densitometric value of 1.8 Kb CYP2E1 transcript/Densitometric value of GAPDH transcript.

DNA extraction, electrophoresis and blot analysis. Genomic DNA was extracted from human tissues as previously described [22]. Ten micrograms of DNA were digested with mentioned restriction enzymes, fractionated on a 1% agarose gel, transferred onto hybond-N filters according to the procedure of Southern [24] and baked for 2 hr at 80°. The blots were prehybridized 2 hr at 68° in 3 × SSC, 5 × Denhardt's, 0.1% SDS and hybridized in the same solution containing 10^5 cpm/mL of the